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THE ANTILYTIC ACTION OF SALT SOLUTIONS AND OTHER SUBSTANCES.*

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INTRODUCTION.

SINCE the earliest bacteriolytic experiments with blood serum, solution of sodium chloride has been used as a diluent; and in the more recent experimental work with lysins of various kinds, as well as with agglutinins and precipitins, the same solution has been used universally for making the necessary dilutions of the mixtures employed. No reason whatsoever has arisen to question in any way the general usefulness of common salt solution for these purposes. Sodium chloride solution of approximately the same concentration as human blood serum is in itself, as a rule, not bacteriolytic or cytolytic; it appears to maintain intact and active the various lysins in normal and immune sera, in venoms, and in other substances; and it does not appear in any noteworthy degree to hinder their union with the different cells and bacteria used.[1] In order to test the action of other chemical substances, especially the various salts (ions) that occur in the normal plasma,

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[1] MADSEN AND ARRHENIUS (*Festskrift ved Indvielsen af Statens Serum-Institut*, Copenhagen, 1902) found that NaCl solution decreases the hemolytic action of bases, and to a less extent of tetanolysin.

upon the bodies concerned in lysis, one can substitute in the ordinary experiments for solution of sodium chloride varying quantities of solutions of other salts of nearly the same concentration $(\frac{m}{8})$ as the so-called physiological salt solution.

In this way important facts might come to light. Substances might be found that diminish or modify in some way the activities of lysins, and the precise mode of action of such substances could be studied. Furthermore, the physico-chemical behavior of the different elements concerned in lysis might perhaps be made clear to some extent, and some light might be thrown upon the intricate mechanisms that lead to diminution or suspension of the lytic properties of serum under various conditions. With these and other considerations in mind, the effect of a number of solutions was tested upon the hemolytic action of human serum on rabbit corpuscles.[1] It was shown that the chlorides of calcium, barium, strontium, magnesium, and the sulphates of magnesium and potassium suspend hemolysis by their action on the complement when 0.2–0.4 c.c. of $\frac{m}{8}$ solution are allowed to act upon 0.2 c.c. of serum. The solutions used being ionizable, it was suggested that this antihemolytic action might be due to the bivalent ions present. It was also pointed out that the same salt solutions suspend the bacteriolytic effect of human serum upon typhoid bacilli. Manwaring[2] then studied the effect of certain salts upon the complement in the serum of a goat immunized with dog blood. He shows that $CaCl_2$, $BaCl_2$, $MgCl_2$, Na_2SO_4, $Na_2C_2O_4$, and $Na_2C_2H_4O_6$ inhibit the lytic action of this serum on dog corpuscles; that the amount of salt necessary to bring about this inhibition varies with the amount of complement in the serum; and that precipitation of the salts from non-hemolytic serum salt mixtures restores the original hemolytic power, which may be destroyed again by heating the fluid to 56° C. for forty minutes. For these reasons Manwaring is inclined to believe that the antilytic action of the salts mentioned is due to the formation of simple salt- or ion-complement compounds that are hemolytically inactive.

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[1] HEKTOEN, *Trans. Chicago Path. Soc.*, 1903, 5, p. 303.

[2] JOUR. OF INFECT. DIS., 1904, 1, p. 112.

In this article we intend to present further and more diversified observations upon the action of a somewhat larger list of salt solutions on the lytic substances in a variety of sera, with the special view to establish to what extent the antilytic action of certain salts holds true of sera in general, and also, if possible, to throw further light upon its mechanism.

BRIEF REVIEW OF NON-SPECIFIC ANTILYSINS.

Various substances other than specific antibodies have been found to diminish or suspend the lytic action of serum and other lysins. Nissen long ago observed that the addition of $MgSO_4$ to plasma in order to hinder coagulation renders the plasma non-bactericidal. Behring[1] found that oxalic acid rendered rat serum harmless for anthrax bacilli because, so he thought, the alkalinity of the serum was reduced. Von Lingelsheim[2] observed that the "alexin" is variously affected by different salts. While KNO_3, when added to rabbit serum in the proportion of 2.5 per cent., had little effect on its destruction of anthrax bacilli, $CaCl_2$ and $(NH_4)_2SO_4$ had a marked inhibitive action. Ferric sulphate, alum, and tannin also reduced the bactericidal power of the serum. He showed, too, that any increase in the salt content of serum reduces its bactericidal power, no matter whether the increase is the result of evaporation or due to the direct addition of salts. Not only an increase in NaCl has this effect, but KNO_3, K_2HPO_4, Na_2SO_4, $(NH_4)_2SO_4$, and $CaCl_2$, when added to rat serum in the proportion of 2 per cent., suspended its bactericidal action on cholera vibrios. Noting the similarity in this effect of salts to their effects upon certain ferments, he suggested that possibly this antibactericidal action is due to the abstraction of water, a certain amount of which is essential for the function of ferments. Markl[3] showed that concentrated salt solutions prevent hemolysis. He regarded this as due to changes in the cell membrane; but Ehrlich and Sachs,[4] while confirming the observation, interpret the antilytic action as due to failure of complement to unite with the amboceptor which is bound by the corpuscles. They point out that Knorr has shown that 10 per cent. NaCl solution prevents the union of tetanus toxin with antitoxin. Here may be mentioned that Bail and Pettersson[5] find that rabbit serum treated with suspensions, or extracts of organs (liver, kidney, muscle), is deprived of its bactericidal action upon anthrax bacilli.

V. Dungern[6] removed the hemolytic complement from serum by shaking it with emulsions of bacteria, with powdered yeast, and with the cells of various organs. Ehrlich and Sachs[7] also found yeast to remove complement from serum. Wilde[1] found that mixing sera with heated bacterial emulsions (anthrax, cholera, typhoid) and with insoluble albuminous substances such as aleuronat removed the bactericidal and hemolytic qualities of the serum. V. Lingelsheim[2] shows that the filtered decoction of Irish moss (carrageen or carrigeen, Chondrus crispus, and Gigartina mamillosa) when added to serum carries down both complement and intermediary body, especially when the alkalescence of the serum is reduced. Hoke[3] shows that organ cells and leucocytes bind the complement in hemolytic serum. Kraus and Lipschütz[4] find that the saline extracts and filtrates of normal organs have the power to bind various hemolysins (megatheriolysin, the lysin of vibrio Naskin, staphylolysin).

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[1] *Gesammelte Abhandl.*, Leipzig, 1893.

[2] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1901, 37, p. 131.

[3] *Ibid.*, 1902, 39, p. 86.

[4] *Berl. klin. Wchnschr.*, 1902, 39, p. 492.

[5] *Centralbl. f. Bakteriol.*, 1903, I. Abt., 34, p. 445 ff.

[6] *Münch. med. Wchnschr.*, 1900, 47, p. 677.

[7] *Berl. klin. Wchnschr.*, 1902, 39, pp. 297, 355.

The antitetanolytic action of horse serum and egg albumin was explained by Arrhenius and Madsen[5] as due to a union between the tetanolysin and the proteids in the serum. Müller,[6] on the other hand, holds that the antihemolytic substances cannot be proteid in character because soluble in alcohol. He favors the idea that the antihemolytic action is due to cholesterin, which has been shown by Ransom[7] to prevent hemolysis by saponin and to give normal serum its antisaponin action, and by Noguchi[8] to be antitetanolytic — a form of antitoxic action which Bashford has designated as pseudoantitoxic. Madsen and Arrhenius found that neutral salts decrease the hemolytic action of bases and to a less extent of tetanolysin. Normal serum and egg albumin also diminish the lytic action of bases. They explain the antilytic action of salts upon bases by assuming that the combination of red corpuscle and base behaves as a weakly dissociated salt, of which only the dissociated molecules are active, and the dissociation of which is decreased by the presence of neutral salt. Bullock[9] found that solutions of $MgSO_4$, $K_2C_2O_4$, and a number of other salts, isotonic with blood corpuscles, greatly reduced the lytic power of immune rabbit serum. Special experiments with $MgSO_4$ seemed to show that lysis was inhibited because the union of complement with amboceptor was prevented.

Wendelstadt[10] shows that glycogen suspends or diminishes hemolysis by its influence on the complement, but only when the amount of amboceptor present is small. For this reason the antihemolytic action of glycogen is demonstrable only in normal serum, and not in immune serum which contains amboceptor in excess. Furthermore, the complement is bound by glycogen only when the latter is added to serum before the proper corpuscles. He states that inulin and peptone diminish hemolysis, the former in normal and immune serum. It is of great interest to note that the antihemolytic action of glycogen may be exercised *in vivo*, the normal hemolytic power returning gradually, reaching the normal in seven days after injecting 3 g. of glycogen into the ear vein of rabbits in three equal doses during twenty-four hours.

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[1] *Ibid.*, 1901, 38, p. 878; *Arch. f. Hyg.*, 1902, 39, p. 404.

[2] *Ztschr. f. Hyg. u. Infektionskrankh.*, 1903, 42, p. 308.

[3] *Centralbl. f. Bakteriol.*, 1903, I. Abt., 34, p. 692.

[4] *Wien. klin. Wchnschr.*, 1903, 16, p. 989.

[5] *Loc. cit.*

[6] *Centralbl. f. Bakteriol.*, I. Abt., 1903, 34, p. 567.

[7] *Deutsche med. Wchnschr.*, 1901, 27, p. 194.

[8] *Centralbl. f. Bakteriol.*, I. Abt., 1902, 32, p. 377.

[9] *Trans. Lond. Path. Soc.*, 1903, 54, p. 260.

[10] *Centralbl. f. Bakteriol.*, 1903, I. Abt., 34, p. 831.

We do not intend to refer in detail to the presence of antilysins in normal sera, but mention must be made of the power of uremic serum to inhibit hemolysis by normal human serum noted by various observers.[1] It would be interesting to learn whether the scope of the inhibition extends farther than to human serum, and, if so, the exact mechanism.

THE ACTION OF SALT SOLUTIONS UPON BACTERIOLYSIS BY NORMAL SERA.

In order to test the effect of salt solutions upon the bacteriolysins in blood serum, the following experiments were made: $\frac{m}{8}$ solutions of the best chemicals obtainable were made in pure, redistilled water, kept in carefully cleansed glass bottles, and used in sterile condition for diluting the serum, as in the usual experiments for the purpose of demonstrating the destructive effect of human serum upon typhoid bacilli. A fixed quantity of twenty-four-hour broth cultures of recently isolated typhoid bacilli, dysentery bacilli, or anthrax bacilli was introduced into a constant quantity of the serum mixture, and the number of bacilli estimated from time to time by counting the colonies that developed on agar plates made with a fixed quantity of the mixtures. The mixtures of serum and solutions of salts were allowed to stand for about one hour before adding the bacilli. This method gives one a fairly satisfactory idea of the fate of the bacilli, but, of course, the chances for error are too great to warrant any attempt to determine accurately minor comparative differences in bactericidal action. The results are set forth in Tables I and II.

Table I shows that even small quantities of many salts are capable of completely inhibiting the lytic action of human serum upon typhoid bacilli and dysentery bacilli. The best effect is obtained with salts of the alkaline earths, but we find here that $MgSO_4$ forms an exception to the rule. $MgSO_4$ is not strongly germicidal, and hence the death of the bacilli introduced into the mixture of serum and a $\frac{m}{8}$ solution of this salt cannot be ascribed to that property of the salt. It was found also that $\frac{m}{8}$ solutions of some salts, as $BaCl_2$, KCNO, $NaHCO_3$, and $Na_2HC_4H_4O_6$, are germicidal when used alone, while a mixture

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[1] CAMUS AND PAGNIEZ, *Soc. de Biol.*, 1901, 53, p. 730; NEISSER AND DOEHRING, *Berl. klin. Wchnschr.*, 1901, 38, p. 593; LACQUER, *Deutsche med. Wchnschr.*, 1901, 27, p. 744; HAHN AND TROMSDORF, *Münch. med. Wchnschr.*, 1902, 49, p. 1454; HEDINGER, *Deutsche Archiv f. klin. Med.*, 1902, 74, p. 24; WENSTRAND, *Trans. Chicago Path. Soc.*, 1903, 5, p. 288.

TABLE I.

The Effect of Salts on Lysis of Typhoid and Dysentery Bacilli by Normal Human Serum.

B. typhosus				B. dysenteriae			
$\frac{m}{8}$ Solutions		At Once	4-5 Hours	$\frac{m}{8}$ Solutions		At Once	4-5 Hours
NaCl	0.8	5,000	0	NaCl	0.8	20,000	0
$CaCl_2$	0.4	3,600	1,800	$CaCl_2$	0.4	20,000	∞
"	0.2	3,800	1	"	0.2	20,000	5,000
$BaCl_2$	0.2	7,000	6,000	$BaCl_2$	0.2	12,000	8,000
$SrCl_2$	0.4	6,000	7,000	$SrCl_2$	0.4	20,000	∞
"	0.2	5,500	5,000	"	0.2	20,000	∞
$MgCl_2$	0.4	4,500	3,500	$MgCl_2$	0.4	12,000	10,000
"	0.2	4,500	0	"	0.2	12,000	1,200
$MgSO_4$	0.4	2,000	0	$MgSO_4$	0.4	12,000	90
"	0.2	2,000	0	"	0.2	12,000	200
$(NH_4)_2SO$	0.4	4,500	200	$(NH_4)_2SO_4$	0.4	12,000	8,000
K_2SO_4	0.4	3,800	0	K_2SO_4	0.4	10,000	10,000
$NaHCO_3$	0.2	4,300	2,500	$NaHCO_3$	0.2	12,000	12,000
"	0.1	3,500	3,500	"	0.1	12,000	220
$Na_3C_6H_5O_7$	0.4	3,500	1,800	$Na_3C_6H_5O_7$	0.4	12,000	∞
"	0.2	4,000	1	"	0.2	12,000	10,000
$Na_2C_2O_4$	0.4	3,800	3,500	$Na_2C_2O_4$	0.4	12,000	15,000
"	0.2	3,500	11	"	0.2	12,000	15,000
$(NH_4)_2C_2O_4$	0.4	5,000	5,500	$(NH_4)_2C_2O_4$	0.4	20,000	∞
"	0.2	5,000	12	"	0.2	20,000	∞
$K_4Fe(CN)_6$	0.4	3,500	4,000	$K_4Fe(CN)_6$	0.4	20,000	∞
"	0.2	3,500	4,000	"	0.2	20,000	∞
$K_6Fe_2(CN)_{12}$	0.4	5,500	55	$K_6Fe_2(CN)_{12}$	0.1	20,000	12,000
KCNO	0.4	3,500	2,500	KCNO	0.4	12,000	10,000
"	0.2	3,500	2,500	"	0.2	12,000	12,000
$NaHC_4H_4O_6$	0.4	3,500	1,800	$NaHC_4H_4O_6$	0.4	12,000	15,000
"	0.2	4,000	4,000	"	0.2	12,000	15,000

Note.—Each tube contained 0.2 c.c. of human serum + 0.1-0.4 c.c. $\frac{m}{8}$ solution of salt + 0.85 per cent. sodium chloride solution enough to make 1 c.c. The experiments represented by the figures in the right-hand columns of both Tables I and II were made as follows: Each tube was inoculated with a loopful of a twenty-four-hour broth culture, and at the same time several plates were made each with one loopful. The average number of colonies in these plates was taken as the number of organisms inoculated into each tube (see "At Once" column). In from four to five hours the entire contents of each tube were plated and the colonies counted in twenty-four hours. In the left-hand columns of Tables I and II each tube was inoculated with a loopful of culture, and two loopfuls of this mixture were plated at once and in four to five hours. Twenty-four plates were also made in many cases, but these showed in reality nothing in addition to what is shown by the five-hour plates; in most instances the bacilli had multiplied so that innumerable colonies developed; ∞ means innumerable.

of one of these solutions and serum forms a good culture medium for the bacteria. Table II shows the same results as those set forth in Table I, but in these experiments rabbit serum and white-rat serum were tested on anthrax bacilli. Only the positive results are given in these tables. $(NH_4)_2SO_4$ and K_2SO_4 have relatively weak actions. Many other salts (KCl, LiCl, $NaClO_3$, $NaC_3H_5O_3$, $Na_2C_4H_4O_6$, Na_2HPO_4, Na_2CO_3, NH_4Cl, $(NH_4)_2CO_3$, $(NH_4)_2HPO_4$, and urea) were experimented with, but as they had no marked effect on bacteriolysis, they were omitted from the tables.

TABLE II.

THE EFFECT OF SALTS ON LYSIS OF ANTHRAX BACILLI BY NORMAL RABBIT AND RAT SERUM.

RABBIT SERUM				WHITE RAT SERUM			
$\frac{m}{8}$ Solutions		At Once	4-5 Hours	$\frac{m}{8}$ Solutions		At Once	4-5 Hours
NaCl		93	0	NaCl		2,100	0
$CaCl_2$	0.4	110	2,000	$CaCl_2$	0.4		2,800
$BaCl_2$	0.4	100	30	$BaCl_2$	0.1		4,000
$SrCl_2$	0.4	115	95	$SrCl_2$	0.4		3,000
“	0.2	110	17	“	0.2		3,500
$MgCl_2$	0.4	90	170	$MgCl_2$	0.4		∞
K_2HPO	0.8	110	150	K_2HPO_3	0.4		6,000
“	0.4	100	140	“	0.2		6,000
$(NH_4)_2SO$	0.4	115	150	$(NH_4)_2SO_4$	0.8		8,000
“	0.2	110	110	“	0.5		8,000
K_2SO	0.8	140	160	K_2SO_4	0.4		35
“	0.4	90	44	“	0.2		60
$MgSO_4$	0.4	200	350	$MgSO_4$	0.8		9,000
“	0.2	210	500	“	0.5		10,000
$Na_3C_6H_5O_7$	0.4	20	900	$Na_3C_6H_5O_7$	0.4		3,000
“	0.2	20	800	“	0.2		3
$Na_2C_2O_4$	0.4	128	140	$Na_2C_2O_4$	0.4		150
“	0.2	150	0	“	0.2		30
$(NH_4)_2C_2O_4$	0.4	80	300	$(NH_4)_2C_2O_4$	0.4		3,000
“	0.2	130	150	“	0.2		4,500
K_4FeCn_6	0.4	120	125	K_4FeCn_6	0.4		1,500
“	0.2	110	18	“	0.2		11
$K_6Fe_2Cn_{12}$	0.4	100	180	$K_6Fe_2Cn_{12}$	0.4		1,200
“	0.1	120	120	“	0.2		90
$NaHC_4H_4O_6$	0.05	180	600	$NaHC_4H_4O_6$	0.05	1,800	4,000

NOTE.—Each tube contained 0.2 c.c. of serum + 0.1–0.8 c.c. $\frac{m}{8}$ solution of salt + 0.85 per cent. sodium chloride solution enough to make 1 c.c.

The addition of these salts to the sera inhibits the bacteriolytic action, but does not destroy that property of the serum, as is shown by the following experiment:

	At Once	3-4 Hrs.
0.2 c.c serum + 1 c.c. NaCl + B. typhosus	1,500	0
0.2 c.c serum + 0.6 c.c NaCl + 0.4 c.c $SrCl_2$ + B. typh.	1,500	1,650
0.2 c.c serum + 4 c.c $SrCl_2$ + 0.4 c.c $Na_2C_2O_4$ + B. typh..	1,450	0

A mixture of $SrCl_2$ and serum makes a good culture medium for typhoid bacilli, but if the $SrCl_2$ is precipitated out of solution by the addition of a proper quantity of $Na_2C_2O_4$, the serum again becomes bactericidal. This result may also be obtained with $CaCl_2$, which may be precipitated with Na_2HPO_4 or $Na_2C_2O_4$, and also with $(NH_4)_2\ C_2O_4$ which may be precipitated with $SrCl_2$ or $CaCl_2$.

Concentrated solutions of sodium chloride, as $\frac{m}{2}$ solution, also inhibit bacteriolysis, but the bacteriolytic power of the serum is again restored by diluting the mixture with distilled water.

These results harmonize very well with those of the similar experiments with hemolytic sera. The same solutions that suspend hemolysis suspend bacteriolysis, as a rule. While it is exceedingly difficult to determine the mechanism of suspension in the case of bacteriolysis, there is reason to believe from analogy that here, too, the complement is the point of attack.

We have been interested especially in some of Behring's early work on immunity to anthrax. Behring[1] investigated the causes of immunity to anthrax on the part of white rats, the serum of which is destructive of anthrax bacilli, but he found that neutralization by means of acids makes it a good culture medium for these organisms. The reduction of the alkalinity during life by the injection of oxalic acid also rendered the serum a good culture medium for anthrax bacilli. He made titrations which show that the serum of white rats is more alkaline than other sera which are not destructive of anthrax bacilli, and concluded from these observations that the anthracidal action of rat serum is due to alkalinity. This conclusion cannot stand, however, because the lytic action of rat serum, like that of other sera, depends upon interaction of amboceptor and complement; rat serum loses its anthracidal power by heating to 58° C. for thirty minutes, but, as its alkalinity is not reduced thereby, the bacteriolytic effect cannot be ascribed to the alkalinity alone. It seems more likely that the suspension of bacteriolysis noted by Behring on the addition to the serum of acids, especially oxalic acid, depended on neutralization of the complement.

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[1] *Loc. cit.*

Having found that so many salts inhibit bacteriolysis by serum, we now attempted to reduce the bacteriolytic power of the serum by injecting salts into animals during life. These experiments are however, still incomplete and unsatisfactory, because it is very difficult to inject enough of any of the salts without causing death of the animals. White rats were injected with 2 c.c $\frac{m}{8}$ solution of $MgCl_2$, and then with anthrax, while one control was injected only with the $MgCl_2$ and another only with anthrax. This experiment was repeated several times, and the rats which had been injected with both $MgCl_2$ and anthrax died first. But the controls which received only anthrax also died, with the exception of one rat. The other controls were not sick. These experiments are unsatisfactory also because the rats at our disposal were less than a year old, and it is well known that young rats are more susceptible to infection with anthrax than old rats.

THE ACTION OF SALT SOLUTIONS UPON HEMOLYSIS BY NORMAL SERA.

In our experiments each tube contained 0.05–0.1 c.c. of serum and 0.2 c.c.–0.4 c.c. of $\frac{m}{8}$ salt solution, with sufficient 0.85 per cent. sodium chloride solution to make 1.0 c.c., and 1.0 c.c. of a freshly made 5 per cent. suspension (NaCl) of carefully washed corpuscles. In all cases the serum solution mixtures were placed at 36° C. for at least one-half hour, and never longer than two hours, before adding the corpuscles. The tubes were then placed at 36° C. for two hours, and subsequently in the ice-box for from sixteen to twenty-four hours. So far as possible, the different sets of experiments—for instance, those dealing with one kind of corpuscle or one kind of serum—were made at the same time with the same sera and the same corpuscular suspensions, all being fresh, so that the conditions were practically uniform, and the results consequently comparable, within certain limits. The reactions of all the mixtures were for the most part alkaline, a few appearing neutral to litmus. The salts used for making the solutions were the purest obtainable, and the water used was first distilled three times over glass. Too much stress cannot be placed upon the necessity of the most careful control of the purity of the salts used in this kind of work. Contamination and alterations in the salt used in making the solutions may prove sources of grave error. In the course of these experiments some sodium fluoride, presumably of reliable make, was found to exercise marked antihemolytic action upon sera. This must have been due to some gross impurity. Unfortunately, it has not been possible to use the same solutions throughout the whole series of experiments. New solutions had to be made from time to time, but the salts used have always been the best obtainable. In order to record the results as accurately as possible, the amount of hemolysis has been put down in percentages determined by means of a colorimetric scale obtained by laking decreasing amounts of corpuscles in 2 c.c. of water, 100 per cent., the starting-point being formed by laking in 2 c.c. of water the corpuscles contained in 1 c.c. of the suspension used in the corresponding experiment; 90 per cent. would be 2 c.c. of water and 10 per cent. less corpuscles, etc.

In the case of certain sera—*e. g.*, dog and cat—considerable difficulty may be met in reading the results on account of cloudiness of the serum, apparently due to the presence in it of finely divided fat. In such cases, as well as in all cases requiring great accuracy, it is well to dilute with salt solution the mixtures as well as fluids composing the colorimetric scale up to several times the original quantities. Smaller differences in color, especially toward the high end of the scale, may now be determined with much greater accuracy. In the case of strongly agglutinative sera the rapid settlement of the corpuscles at the bottom of the tubes may interfere somewhat with the lytic action, which always requires some time for full development, especially when small quantities are used of but moderately active serum.

In order to obtain as accurate an idea as possible of the degree or strength of antihemolytic action of the salts studied in these experiments, the quantities of serum employed have in most cases barely been sufficient to cause complete laking of 1.0 c.c. of the

5 per cent. corpuscular suspension. In the case of sheep, rabbit, and swine sera, however, the hemolytic power of the quantities employed in the experiments fell far short of this standard.

Table III gives a general idea of the extent of our observations, so far as concerns the variety of sera and corpuscles used, and also the general nature of the results obtained with the salts to which it is desired to direct special attention at this time. We have not tabulated the results of a number of experiments with KCl, NaFl, LiCl, NH_4Cl, NaI, NaBr, $NaNO_2$, $NaNO_3$, $NaClO_3$,

TABLE III.

THE ACTION OF SALT SOLUTIONS UPON HEMOLYSIS BY VARIOUS SERA.

Solutions $\left(\frac{m}{8}\right)$	Human Corpuscles + Serum of					Rabbit Corpuscles + Serum of								
	Goat 0.1	Sheep 0.15	Beef 0.1	Dog 0.1	Rab't 0.1	Goat 0.1	Sheep 0.15	Beef 0.1	Dog 0.1	Man 0.1	Horse 0.2	Cat 0.1	Chicken 0.2	Swine 0.1
NaCl	80	25	80	60	30	100	100	100	80	80	100	80	100	20
$CaCl_2$ 0.2	20	0	5	10	0	25	0	0	0	0	10	0	0	0
$BaCl_2$ 0.2	0	0	0	0	0	10	0	0	0	0	0	0	20	0
$SrCl_2$ 0.2	0	0	0	0	0	10	0	0	0	0	0	0	20	0
$MgCl_2$ 0.4	25	0	15	0	0	20	20	10	0	10	10	50	35	0
K_2SO_4 0.4	10	0	30	15	30	85	10	40	10	45	0	35	100	5
$MgSO_4$ 0.4	0	0	10	15	5	65	20	40	30	45	20	50	100	5
$(NH_4)_2CO_3$ 0.2	30	0	0	0	5	20	100	45	35	40	50	50	40	100
$NaHCO_3$ 0.2	15	0	0	0	10	10	100	30	55	50	50	70	0	50
$Na_3C_6H_5O_7$ 0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$Na_2C_2O_4$ 0.2	0	0	0	0	0	10	0	5	0	0	0	0	5	0
$(NH_4)_2C_2O_4$ 0.2	0	0	0	0	0	30	5	12	0	0	0	0	0	5
$K_4Fe(Cn)_6$ 0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$K_6Fe_2(Cn)_{12}$ 0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Solutions $\left(\frac{m}{8}\right)$	Guinea-Pig Corp. + Serum of					Ch'ck'n Corp. + Ser. of		Horse Corp. + Serum of			W. Rat Corp. + Ser. of		Dog Serum 0.1 + Corp. of			
	Goat 0.1	Beef 0.1	Sheep 0.15	Swine 0.1	Dog 0.05	Goat 0.1	Dog 0.1	Goat 0.1	Man 0.15	Dog 1.1	Chicken 0.2	Dog 0.3	Beef	Sheep	Swine	Goat
NaCl	100	100	100	40	100	80	100	100	100	100	100	70	90	100	90	90
$CaCl_2$ 0.2	35	5	5	10	20	0	10	0	0	0	40	10	10	10	0	0
$BaCl_2$ 0.2	0	0	2	10	10	0	0	0	0	0	30	10	0	10	0	0
$SrCl_2$ 0.2	5	0	3	0	10	5	10	0	0	5	50	20	0	10	0	0
$MgCl_2$ 0.4	50	50	30	40	20	50	50	50	10	70	60	70	10	30	10	0
K_2SO_4 0.4	50	80	10	40	90	40	100	50	40	100	75	0	40	30	100	90
$MgSO_4$ 0.4	60	100	50	40	90	40	100	40	10	10	90	20	40	30	100	30
$(NH_4)_2CO_3$ 0.2	10	30	5	40	70	50	30	100	100	100	20	30	25	30	10	10
$NaHCO_3$ 0.2	10	20	20	20	60	50	10	100	100	100	50	10	20	20	10	0
$Na_3C_6H_5O_7$ 0.2	0	0	0	10	20	0	25	0	0	5	0	20	0	10	5	0
$Na_2C_2O_4$ 0.2	20	30	5	5	30	10	10	5	0	0	10	20	0	0	15	0
$(NH_4)_2C_2O_4$ 0.2	20	20	25	10	10	20	10	0	30	20	20	30	5	20	20	0
$K_4Fe(Cn)_6$ 0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$K_6Fe_2(Cn)_{12}$ 0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

NOTE.—In all the experiments tabulated in this article 0 means no recognizable hemolysis. It is probable that in all cases a small degree of lysis takes place.

Na_2CO_3, KCN, and several other substances (urea, oleate, lactate, etc.), because of the general absence in most instances of all, or at least of any marked, antilytic effect on the part of their solutions $(\frac{m}{8})$ in the proportions of 0.2 c.c. to 0.1 c.c. or so of serum. It is possible, of course, that some of the solutions may prove antilytic in certain cases and in larger quantities of $\frac{m}{8}$ solutions than those here used. In high concentrations — $\frac{m}{2}$ and $\frac{m}{3}$ — NaCl, KCl, as well as $NaNO_3$, $NaNO_2$, and $KC_2H_3O_2$, and probably also many others, prevent lysis by serum.[1] It is noteworthy that the corpuscles absorb amboceptors even in the concentrated solutions, observed also by Ehrlich and Sachs, and a few preliminary experiments indicate that the hemolytic power (as well as the bacteriolytic) of sera treated with such solutions is restored upon diluting the mixtures; evidently the antilytic action of the salts $(\frac{m}{8}$ solutions$)$ in Table III depends on other factors than that of concentration.

There are, however, many salts other than those mentioned in Table III that will diminish or suspend lysis by serum in $\frac{m}{8}$ solutions; *e. g.*, tartrate (shown by Manwaring to be antilytic for goat serum, and by us to be antibacteriolytic), salicylate, benzoate, thiosulphate, and tungstate of sodium. These have not been included in a sufficient number of experiments to warrant further consideration at this time. Numerous experiments have been made with K_2HPO_4, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, KCNO and KCNS, but, on account of variability, the results are as yet not ripe for full discussion. $CaCl_2$, $BaCl_2$, $SrCl_2$, $Na_3C_6H_5O_7$, $Na_2C_2O_4$, $(NH_4)_2C_2O_4$, $K_4Fe(CN)_6$, and $K_6Fe_2\,(CN)_{12}$ constantly and completely, or nearly completely, suspend hemolysis by normal sera under the conditions peculiar to these experiments. It should be remembered that normal serum may vary in its hemolytic power, so that our results must not be accepted in too absolute a sense.

$MgCl_2$, K_2SO_4, and $MgSO_4$ have proved rather disappointing, because the earlier experiments with human serum and rabbit corpuscles showed them to possess decided antilytic properties, which they, however, have not maintained in the work with other sera. It is possible that more concentrated (because older) solutions were used at first. Impurities may also have been present. These salts have also only a weak antibacteriolytic action.

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[1] See also von Lingelsheim's and Markl's experiments, *loc. cit.*

INHIBITION BY SALT SOLUTIONS OF THE ACTIVATION BY GUINEA-PIG SERUM OF THE HEMOLYTIC AMBOCEPTORS IN COBRA VENOM.

Kyes[1] has shown that the hemolytic amboceptor of cobra venom is activated by lecithin, with which the amboceptor may form a crystallizable chemical compound—a discovery of the greatest significance to the study of lysins. Cobra venom is also activated by certain constituents of normal serum that in every way correspond to the complements in the usual sense of the word. The careful experiments of Kyes and Sachs[2] show conclusively that the complement in guinea-pig serum which activates cobra venom hemolytically is different and distinct from lecithin. For the purpose of studying the actions of antilytic salt solutions upon lecithin and guinea-pig serum in their rôle as activators of the hemolytic amboceptors of cobra venom, the following experiments were made (Table IV).

The recorded quantities of lecithin and guinea-pig serum were mixed with salt solutions, made up to 1 c.c. by means of sodium chloride solution, and placed in the incubator at 37.5° C. for two hours; at the end of this time the venom and corpuscles were added, the tubes returned to the incubator for two hours, and then placed in the ice-box over night. The experiment was repeated several times, with practically uniform results. In some of the mixtures containing salt solutions and lecithin there was a noticeable delay in the laking; *e. g.*, the $CaCl_2$, $BaCl_2$, $MgSO_4$, $NaHCO_3$, $K_4Fe(CN)_6$, and $K_6Fe_2(CN)_{12}$ tubes. Certain beef corpuscles are more susceptible to laking by the ammonium salts used, and by sodium bicarbonate, than others. In the experiments recorded in Table IV perfectly fresh corpuscles were used, and in this case only the $(NH_4)_2CO_3$ and $NaHCO_3$ solutions (0.5 c.c.+0.5 c.c. NaCl sol.+1 c.c. 5 per cent. suspension of corpuscles) caused any marked laking—about 40 per cent. Other experiments would have to be made in order to show to what

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[1] *Berl. klin. Wchnschr.*, 1903, 40, p. 956.

[2] *Ibid.*, 1903, 40, p. 21.

TABLE IV.

ACTION OF SALT SOLUTIONS ON THE LYSIS OF BEEF CORPUSCLES BY COBRA VENOM ACTIVATED (*a*) BY LECITHIN (*b*) BY GUINEA-PIG SERUM.

SOLUTIONS $\left(\frac{m}{8}\right)$	SUSPENSION OF BEEF BLOOD (5 PER CENT.), 1 C.C., AND COBRA VENOM, 0.0002 C.C.+		WASHED, NON-HEMOLYZED, VENOM-SERUM CORPUSCLES +	
	(*a*) Lecithin, 0.0001 c.c.	(*b*) Guinea-Pig Serum 0.2 c.c.	(*a*) Lecithin, 0.0001	(*b*) Guinea-Pig Serum, 0.1 c.c.
NaCl	100	100		
$CaCl_2$ 0.5........	100	5	+	+
$BaCl_2$ 0.5........	50	4	+	+
$SrCl_2$ 0.5........	100	10	+	+
$MgCl_2$ 0.5........	100	20	+	+
K_2HPO_4 0.5........	100	100		
$(NH_4)_2HPO_4$ 0.5........	100	100		
$(NH_4)_2SO_4$ 0.5........	100	100		
K_2SO_4 0.5........	100	70		
$MgSO_4$ 0.5........	100	10	+	+
$(NH_4)_2CO_3$ 0.5........	100	100		
$NaHCO_3$ 0.5........	100	90		
$Na_3C_6H_5O_7$ 0.5........	20	0	+	+
$Na_2C_2O_4$ 0.5........	10	5	+	+
$(NH_4)_2C_2O_4$ 0.5........	100	20	+	+
$K_4Fe(Cn)_6$ 0.5........	100	0	+	+
$K_6Fe_2(Cn)_{12}$ 0.5........	+*	0		
KCNO 0.5........	100	100		
KCNS 0.5........	100	100		

*Color masked by the color of the salt solution.

degree, if any, these solutions prevent lysis by venom + guinea-pig serum or lecithin.

Both lecithin and guinea-pig serum cause hemolysis when added to the washed intact corpuscles in those cases in which the hemolytic action of venom + serum were suspended wholly or in part by salt solutions, as shown in Table IV. These corpuscles were washed twice in large quantities of NaCl solution and resuspended in 2 c.c. of the latter. This indicates that the corpuscles were venomized in the serum-salt-venom mixtures. Only minute traces of laking developed in the control experiments in which the corpuscles were treated similarly with serum-salt mixtures and venom, then washed, and resuspended without the addition of the lecithin or serum. In the case of the potassium ferricyanide experiment, however, the corpuscles underwent complete laking in the course of the washing.

These results support the conclusions of Kyes and Sachs that lecithin and serum complements in the usual sense are different and distinct substances. The fact that sodium citrate and sodium oxalate greatly diminish lysis by venom and lecithin is not without interest. (It may be recalled that Kyes and Sachs found that guinea-pig serum contains a substance which inhibits activation of cobra venom by lecithin. The inhibitory effect is exercised by so small quantities of serum that the complement in the latter does not suffice to activate the venom.) But the results shown in Table IV also support the conclusion that the antilytic action of salt solutions is due to their effect upon the complement.

THE ANTILYTIC ACTION OF DISINTEGRATED TYPHOID BACILLI.

In view of the bactericidal effect of human serum on typhoid bacilli, the development of typhoid fever, in which the bacillemia of recent investigations must play an important rôle, appears at first sight somewhat puzzling, if we assume that the blood has the same action *in vivo* as the serum *in vitro*. The full discussion of this problem does not fall within the scope of this paper. The similar problem of anthrax has been studied recently, with interesting results, by Bail and Pettersson.[1]

The possibility that the disintegration of typhoid bacilli might give rise to antilytic substances led to the following experiments:

A large excess of young typhoid bacilli was added to a quantity of human serum, incubated for eight hours, diluted with an equal volume of salt solution, and passed through a porcelain filter. This serum was now no longer bactericidal, but had decided antibactericidal properties, as is shown by the following experiment:

Filtrate	Human Serum	Typhoid Bacilli	
		At Once	6 Hours
1 c.c.	0.1 c.c.	5,000	0
1 c.c.	0.05 c.c.	5,000	10,000
1 c.c.	0.025 c.c.	5,000	∞
NaCl Sol.			
1 c.c.	0.1 c.c.	5,000	0
1 c.c.	0.05 c.c.	5,000	0
1 c.c.	0.025 c.c.	5,000	0

[1] *Loc. cit.*

The objection might be raised that the difference in bacteriolysis in these two sets of tubes is due to the fact that the exhausted serum forms a good nutritive medium. This objection is rendered improbable, however, by the fact the exhausted serum has also antihemolytic properties in that it protects guinea-pig corpuscles against human serum.

The products of autolysis of typhoid bacilli also have antilytic properties. Following the method of Neisser and Shiga, twenty-four-hour growths of typhoid bacilli on glycerin agar slants were suspended in salt solution (10 c.c. for each tube), the suspension heated for one hour at 60° C., incubated at 36° C. for forty-eight hours, and passed through a porcelain filter. The colorless, transparent fluid thus obtained is neutral in reaction. In quantities of 1–0.1 c.c. to 0.1–0.2 c.c. of serum this fluid has been found to suspend lysis of sheep corpuscles by immune goat serum and of sensitized sheep corpuscles by normal goat serum; of rabbit corpuscles by human serum; of human, guinea-pig, and beef corpuscles by dog serum; and of human, guinea-pig, and beef corpuscles by sheep serum. Not all fluids of this kind have the same antilytic strength, and the lytic power of the different sera also varies considerably. Beef serum has not been inhibited by this fluid, heating of which to the boiling-point has not affected the antilytic action.

The antilytic action of the soluble products of autolysis of typhoid bacilli seems to be due to the effect upon the complement, because guinea-pig corpuscles are sensitized in dog serum mixture and sheep corpuscles in the immune goat serum mixture, as shown by prompt lysis after washing the corpuscles and adding the proper complements in small doses (0.05 normal guinea-pig serum in the first case and 0.005 normal guinea-pig serum or 0.05 normal goat serum in the second case).

Soluble products of autolysis of typhoid bacilli also hinder bacteriolysis, as shown by the following experiment:

	At Once	4 Hrs.
Autolytic fluid 1 c.c. + human serum + typhoid bacilli.....	3,600	1,500
NaCl solution 1 c.c. + human serum + typhoid bacilli......	3,500	0

The results of these experiments may be taken to indicate that in typhoid infections not only is complement used up, in the course of the bacteriolysis, which no doubt is going on, but that a certain amount is also neutralized by the soluble products of disintegration of typhoid bacilli.

Wilde[1] interpreted the removal of lytic properties of serum by dead bacteria as evidence against the amboceptor-complement nature of lysins. Closer analysis of his results might have shown, however, that the opposite conclusion would have been correct.[2]

THE MODE OF ACTION OF ANTILYTIC SALT SOLUTIONS AND THEIR RELATIONS TO THE COMPLEMENTS.

There can be no question that the antilytic salts now considered prevent lysis by virtue of their action on the complement. This must be so (1) because the corpuscles freely take up the intermediary bodies in the various non-lytic serum salt mixtures; (2) because precipitation of the inhibiting salt restores the lytic property of the complement; (3) because of the interesting quantitative relationships between complements and antilytic salt solutions.

1. Repeated experiments show that corpuscles become sensitized,[3] *i. e.*, absorb intermediary body, in the non-lytic serum salt mixtures. The treated corpuscles were washed two or three times in sodium chloride solution, and then subjected to the action of pure complement. In all cases complete or marked lysis resulted promptly with minimum doses of complement, thus showing that the absence of lysis in the mixtures is not due to *Komplementoidverstopfung* (Ehrlich and Sachs) of the amboceptor. In the controls of washed treated corpuscles suspended in sodium chloride solution without the addition of complement no laking takes place. (Ferrocyanide and ferricyanide of potassium seem to render guinea-pig corpuscles susceptible to laking under these circumstances.) It may be emphasized here that in no case has the treatment of sensitized corpuscles with antilytic solutions inter-

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[1] *Loc. cit.*

[2] We have found since writing the above that Wright and Windsor (*Jour. of Hyg.*, 1902, 2, p. 385) observed that digestion of serum with typhoid or cholera cultures produces antibactericidal substances.

[3] Throughout this article the word "sensitized" is used only in the sense here indicated, and not in the sense in which some French authors speak of "sensibilization."

fered with lysis after careful washing and the addition of complement.

It has not been possible to go through the entire list of experiments in the manner just indicated, but it has been determined that absorption of amboceptor in non-lytic serum salt mixtures takes place in the case of human corpuscles treated with beef, sheep, and goat serum (washing and adding corresponding complements); of rabbit corpuscles treated with human serum and immune guinea-pig serum; of sheep corpuscles treated with immune goat serum, and of guinea-pig corpuscles treated with dog, beef, and horse serum. The guinea-pig corpuscles treated without hemolysis by dog and beef serum were laked, after washing, by adding 0.1 c.c. of normal guinea-pig serum, and those not laked by goat serum were laked after washing and adding 0.1 c.c. horse serum. These results, taken in connection with Manwaring's observations to the same effect, as regards dog corpuscles and immune goat serum, show that the salts suspend lysis by action on the complement, which is prevented from joining the amboceptor. That corpuscles absorb intermediary bodies in non-lytic mixtures of sera and salt solutions is shown also by simply adding to such mixtures containing intact corpuscles a surplus of pure complement, *e. g.*, normal guinea-pig serum, and normal goat serum to rabbit and sheep corpuscles treated respectively with immune guinea-pig serum and goat serum, which is followed by prompt laking, the anticipated result, if the conclusion that the antilytic salts act on complement is well founded.

2. Manwaring found that precipitation of inhibiting salts restores the lytic action of complement in goat serum. We have verified this fact for a number of sera, normal as well as immune, so far as inhibition by $CaCl_2$, $BaCl_2$, $SrCl_2$, and $Na_2C_2O_4$ is concerned, but attempts to liberate the complements in ferro- and ferricyanide mixtures by precipitation with equivalent quantities of $\frac{m}{8}$ solutions of nickel and copper chloride met with failure. As already pointed out, the bacteriolytic action of serum is also restored by precipitation of the antisalt—a definite indication that here, too, the complement is the point of attack.

In some instances active hemolytic complement was liberated from mixtures containing neutralizing doses of $CaCl_2$, $BaCl_2$, and $SrCl_2$, by precipitation of the latter several days after the complement had disappeared from the control specimens of untreated serum. Here, one might say, it would seem as if the salt solutions rendered the complement more resistant to the influences that lead to its so-called spontaneous degeneration; or that the ion or salt-complement compound is more stable than the form in which complement naturally occurs in serum.

3. In case the solutions prevented lysis by direct action upon the bodies of the corpuscles (or the bacteria), one would hardly expect any such accurate quantitative relationship between the solutions and the complements as is the actual case. The protection against lysis by a given quantity of $\frac{m}{8}$ solution is quickly overcome when the amount of serum or complement exceeds a certain definite limit, indicating that there is nothing in the way of free complement directly uniting with the sensitized corpuscle and causing its lysis. While 0.2 c.c. of $CaCl_2$ or of $Na_3C_6H_5O_7$ protect guinea-pig corpuscles against lysis by 0.1 c.c. of goat serum, prompt laking occurs if to this mixture is added 0.05 c.c. of dog serum. The same result is obtained if we reverse the sera, indicating again that the corpuscles are as susceptible as ever to active serum. Similar experiments with sheep corpuscles sensitized with heated immune goat serum and the complement in normal goat serum and guinea-pig serum give the same result.

Here may be mentioned again that the activation of cobra venom by guinea-pig serum is prevented by a number of salt solutions, which, however, have no such action on lecithin—a phenomenon most readily explained as due to the direct action of the salts on the guinea-pig complement.

Manwaring also finds that the amount of salt necessary to prevent lysis varies directly with the amount of complement in the serum used. Tables V and VI record more recent experiments with other sera, normal as well as immune, the results of which show that decreasing amounts of antilytic solutions give increasing lysis with the same quantity of complement or serum; that increasing quantities of complement give increasing lysis with the same amount of salt solution; and finally that multiple proportions of the amount of complement and salt in a given mixture maintain about the same lytic value. The quantitative relationships are of such a character that there seems to be good reason to believe that in their mutual action these substances follow demonstrable physico-chemical laws.

TABLE V.

Quantitative Relations between Certain Antilytic Solutions and Dog Serum.

DOG SERUM AND GUINEA-PIG CORPUSCLES.

$CaCl_2$			$NaHCO_3$			$Na_3C_6H_5O_7$			$Na_2C_2O_4$			$K_4Fe(CN)_6$		
Amount of			Amount of			Amount of			Amount of			Amount of		
Sol.	Com.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis
0.24	0.05	10	0.24	0.05	20	0.24	0.05	15	0.24	0.05	0	0.15	0.05	15
0.21	0.05	15	0.21	0.05	20	0.21	0.05	25	0.21	0.05	21	0.12	0.05	30
0.18	0.05	20	0.18	0.05	28	0.18	0.05	30	0.18	0.05	35	0.09	0.05	40
0.15	0.05	28	0.15	0.05	35	0.15	0.05	42	0.15	0.05	43	0.06	0.05	55
0.12	0.05	40	0.12	0.05	50	0.12	0.05	48	0.12	0.05	50	0.03	0.05	80
0.18	0.07	30	0.18	0.07	40	0.21	0.07	50	0.24	0.07	35	0.12	0.07	60
0.18	0.09	60	0.18	0.09	70	0.21	0.09	70	0.24	0.09	50	0.12	0.09	70
0.36	0.1	15	0.36	0.1	25	0.42	0.1	20	0.48	0.1	5	0.24	0.1	5
0.72	0.2	5	0.72	0.2	35	0.84	0.2	40	0.96	0.2	5	0.48	0.2	5

DOG SERUM AND SHEEP CORPUSCLES.

$CaCl_2$			$NaHCO_2$			$Na_3C_6H_5O_7$			$Na_2C_2O_4$			$K_4Fe(CN)_6$		
Amount of			Amount of			Amount of			Amount of			Amount of		
Sol.	Ser.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis	Sol.	Ser.	Lysis
0.24	0.05	10	0.24	0.05	15	0.24	0.05	25	0.15	0.05	10	0.15	0.05	16
0.21	0.05	10	0.21	0.05	18	0.21	0.05	27	0.12	0.05	30	0.12	0.05	30
0.18	0.05	15	0.18	0.05	30	0.18	0.05	30	0.09	0.05	40	0.09	0.05	38
0.15	0.05	20	0.15	0.05	36	0.15	0.05	32	0.06	0.05	45	0.06	0.05	40
0.12	0.05	25	0.12	0.05	40	0.12	0.05	35	0.03	0.05	55	0.03	0.05	50
0.18	0.07	20	0.28	0.07	15	0.21	0.07	20	0.24	0.07	15			
0.18	0.09	30	0.28	0.09	30	0.21	0.09	30	0.24	0.09	25			
0.36	0.1	25	0.36	0.1	5	0.42	0.1	0	0.48	0.1	0			
0.72	0.2	5	0.72	0.2	5	0.84	0.2	0	0.96	0.2	0			

When added to sera, some salt solutions may form by dissociation substances in themselves lytic for *certain* corpuscles. Here some laking results in spite of antilytic action of the salt upon the serum. Our experiments show that in certain instances an excess of salt solutions, especially of those containing NH_3 and OH ions, above that necessary to prevent hemolysis undoubtedly causes considerable laking of some corpuscles. This is especially true of carbonate and oxalate of ammonium, and to a less degree of bicarbonate of sodium. Thus 0.1 c.c. of $\frac{m}{8}$ solution of ammonium carbonate prevents or greatly reduces lysis of horse corpuscles by 0.1 c.c. of goat serum, and of horse and goat corpuscles by 0.1 c.c. of dog serum; but if the quantity of solution is increased, marked laking may result (Table VII). Similar illustrations are furnished also by rabbit corpuscles and sheep corpuscles. In our experiments the fact, emphasized by Arrhenius and Madsen, that the corpuscles bind a certain amount of NH_3 before laking occurs, is noticeable. It seems also that serum—heated as well as unheated—may interfere with the laking by ammonia. It is quite likely that the extent to which ammonia may be bound by different corpuscles and sera is subject to considerable variation, and that this may be a factor in the variability of the results

TABLE VI.

QUANTITATIVE RELATIONS BETWEEN CERTAIN ANTILYTIC SOLUTIONS AND COMPLEMENT.

SENSITIZED SHEEP CORPUSCLES + NORMAL GOAT SERUM.

$CaCl_2$			$BaCl_2$			$NaHCO_3$			$Na_3C_6H_5O_7$			$Na_2C_2O_4$		
AMOUNT OF			AMOUNT OF			AMOUNT OF			AMOUNT OF			AMOUNT OF		
Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis
0.3	0.1	10	0.3	0.1	10	0.3	0.1	20	0.3	0.1	5	0.3	0.1	20
0.25	0.1	22	0.25	0.1	20	0.25	0.1	20	0.25	0.1	5	0.25	0.1	30
0.2	0.1	50	0.2	0.1	40	0.2	0.1	20	0.2	0.1	5	0.2	0.1	50
0.15	0.1	100	0.15	0.1	52	0.15	0.1	28	0.15	0.1	30	0.15	0.1	65
0.1	0.1	100	0.1	0.1	100	0.1	0.1	37	0.1	0.1	50	0.1	0.1	100
						0.1	0.05	20	0.1	0.05	10			
						0.2	0.1	20	0.2	0.1	10			

SENSITIZED SHEEP CORPUSCLES + NORMAL GUINEA-PIG SERUM.

$CaCl_2$			$NaHCO_3$			$Na_3C_6H_5O_7$			$Na_2C_2O_4$			$K_4Fe(CN)_6$		
AMOUNT OF			AMOUNT OF			AMOUNT OF			AMOUNT OF			AMOUNT OF		
Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis	Sol.	Com.	Lysis
0.15	0.005	15	0.15	0.005	20	0.15	0.005	20	0.15	0.005	30	0.06	0.005	15
0.12	0.005	25	0.12	0.005	35	0.12	0.005	25	0.12	0.005	40	0.04	0.005	25
0.09	0.005	30	0.09	0.005	50	0.09	0.005	30	0.09	0.005	60	0.02	0.005	35
0.06	0.005	40	0.06	0.005	100	0.06	0.005	35	0.06	0.005	100	0.06	0.0075	30
0.15	0.0075	35	0.12	0.0075	100	0.03	0.005	40	0.12	0.0075	100	0.06	0.01	38
0.15	0.01	40	0.12	0.01	100	0.12	0.0075	30	0.12	0.01	100	0.15	0.0075	5
0.45	0.0075	15	0.45	0.0075	20	0.12	0.01	35	0.45	0.0075	20	0.2	0.01	5
0.6	0.01	15	0.6	0.01	20	0.45	0.0075	20	0.6	0.01	20			
						0.6	0.01	20						

NOTE.—Special mention must be made of agglutination as a source of error, especially in experiments with immune serum. The tendency to agglutination not only makes it difficult to secure an equal distribution of the corpuscles in the suspension, but the rapid settlement of the corpuscles may interfere more or less with lysis.

in the antilytic experiments with many ammonium salts. $CaCl_2$ solution is also lytic for some corpuscles, unless bound or neutralized by substances in the serum (complement?), as observed also by Manwaring—a fact that hardly would harmonize with the idea that $CaCl_2$ prevented lysis by direct action on the corpuscles.

The practically uniform antihemolytic (and antibacteriolytic) actions of $CaCl_2$, $BaCl_2$, $SrCl_2$, $Na_3C_6H_5O_7$, $Na_2C_2O_7$, $(NH_4)_2C_2O_4$, $K_4Fe(CN)_6$, and $K_6Fe_2(CN)_{12}$, upon all sera examined speaks strongly in favor of great similarity of nature and structure of various complements in various sera and in the same serum. Whether it is possible on the strength of the data at hand in regard to certain salts with variable antihemolytic action (see Table III) to make out any real quantitative differences in complements is very doubtful, because our experiments with normal sera have been made on the same general plan, without any attempts to determine the exact lytic dose of complement for the amount of amboceptor in each case.

TABLE VII.

LYSIS BY EXCESS OF ANTILYTIC DOSE OF $(NH_4)_2CO_3$ AND $(NH_4)_2C_2O_4$.

SOLUTIONS $\left(\frac{m}{8}\right)$		SHEEP CORPUSCLES +		GOAT CORP. +	HORSE CORPUSCLES +		GUINEA-PIG CORP. +	RABBIT CORP. +
		Dog Ser., 0.05	Immune Goat Ser., 0.1	Dog Ser., 0.1	Dog Ser., 0.1	Goat Ser., 0.1	Dog Ser., 0.05	Immune Guinea-Pig Ser., 0.05
NaCl		100	100	100	100	100	100	100
$(NH_4)_2CO_3$	0.5	35	20	20	100	60	75	100
"	0.4	25	10	0	85	40	70	100
"	0.3	5	0	0	30	30	20	70
"	0.2	5	0	0	20	20	20	40
"	0.1	25	5	10	30	0	30	0
"	0.05	50	30	30	30	5	70	20
$(NH_4)_2C_2O_4$	0.5	35	40	60				
"	0.4	25	40	45				
"	0.3	20	20	10				
"	0.2	20	5	0				
"	0.1	35	40	0				
"	0.05	100	80	80				

Were this done, and the antilytic effect of varying quantities of the solutions then determined, a much truer estimate would be possible of their real value. In other words, accurate quantitative experiments, which can be made best with immune sera, are necessary to determine the exact antilytic value of a salt. Inasmuch as many sera contain more than one complement, and inasmuch as the antisalts, as a rule, seem to act on all complements in a serum, it becomes clear that, in order to determine with utmost exactness the precise antilytic dose of a solution, it would be necessary to have a serum with but one complement.

No doubt the relative amounts of amboceptor and complement vary in the sera of different animals, and even in the same animal at different times. Now von Dungern, Gruber, and especially Morgenroth and Sachs,[1] show that in the presence of large amounts of amboceptors relatively small amounts of complements suffice for lysis. Hence in normal sera with high complement content enough free complement may remain in some serum salt mixtures to cause more or less laking, depending on the amount of amboceptor, and thus variation in the relative amount of amboceptor and complement may help to account for variations in the antilytic action of salts. In the course of the experiments with sensitized corpuscles and complement (Table VI), Morgenroth and Sachs's observations on the effect of larger doses of amboceptor in diminishing the amount of complement necessary for lysis could be confirmed frequently. Owing to this relationship between complement and amboceptor, it is difficult to obtain uniform results in experiments with antilytic salts and immune sera.

Of the many other factors that would have to be considered in any attempt to discuss more fully the variations in the antilytic action of some salts we would mention: the conditions that influence the dissociation of the salts; the variations in the susceptibility of corpuscles to various forms of laking; in the case of the ammonium salts, the variations in the amounts of lytically active ammonia set free; and variations in the relative amounts of different complements in a given serum, as well as in the affinity for complement on the part of amboceptors.

As already pointed out, the interesting fact that complements in general are neutralized by certain salts indicates a close relationship in the chemico-physical nature of complements. Their ready response to this action of salts is in marked contrast to the behavior of the intermediary bodies, which appear wholly unaffected by the same salts in the quantities necessary to counteract the complements. Perhaps this important point of distinction may prove of service in the further study of these bodies.

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[1] *Berl. klin. Wchnschr.*, 1902, 39, pp. 517.

At this time we do not wish to enter upon any extended discussion of the interesting, but difficult, problem of the exact mode of neutralization of complements by certain salts—whether the function of the atomic weight, the valency, the nature of the electrical charge,[1] or solution tension of the ions.[2]

CONCLUSIONS.

In small doses of $\frac{m}{8}$ solutions many salts prevent lysis of red corpuscles and of bacteria by various sera. The same salts inhibit the activation of cobra venom by guinea-pig serum.

The soluble substances of disintegrated typhoid bacilli have antibacteriolytic and antihemolytic properties.

The antilytic salts here considered prevent hemolysis, and probably also bacteriolysis, by virtue of their action on the complements, (1) because in non-lytic serum salt mixtures corpuscles freely take up amboceptors whose complementophile groups remain free; (2) because precipitation of antisalts restores lytic property of complement; (3) because of the quantitative relationship between complements and antisalts.

It is probable that in their reactions complements and antilytic salt solutions follow simple physico-chemical laws.

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[1] The following extract from one of LOEB's articles (*Popular Science Monthly*, 1902, 61, p. 80) may be given as representing his conception of the mode of physiological action of electrically charged molecules: "The fact that ions may act toxically through their electrical charges, and that ions with the opposite charge may act antitoxically, may open a new and very fertile field for pathology and therapeutics. . . . . Two years ago I pointed out that we must realize the existence of physiologically balanced salt solutions, that means salt solutions in which the ions are so combined that the toxic effects of the one are counteracted by the antitoxic effect of some other ion. Any disturbance in the right proportions of monovalent ions and ions of higher valency must lead to more or less pronounced modifications of the life phenomena." In connection with this it is interesting to note that VON BEHRING (*Beiträge zur exp. Therapie*, 1904) attempts to explain the neutralization of tetanus toxin by antitoxin by the assumption that + energy is neutralized by – energy, without there necessarily being any chemical union of the antitoxin with the toxin.

[2] A. P. MATHEWS, *Amer. Jour. of Physiol.*, 1904, 10, p. 290.

That complements generally are neutralized by the same salts indicates a close relationship of their chemico-physical properties.

So far as their functions in lysis are concerned, amboceptors appear to remain unaffected by antilytic salts in quantities sufficient to neutralize the complements.

The susceptibility of complements to the influence of various non-specific anticomplements—*e. g.*, salts, glycogen (Wendelstadt), products of disintegrated typhoid bacilli—suggests that neutralization of complements may play an important rôle in certain infections.